Journal of Chromatography B, 875 (2008) 459–464

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of glycemic monitoring marker 1,5-anhydroglucitol in plasma by liquid chromatography-electrospray tandem mass spectrometry

Shuijun Li[a,b], Xiaoxiang Heng[c], Hongguang Sheng[d], Yiping Wang[b], Chen Yu[a,*]

[a] Central Laboratory, Shanghai Xuhui Central Hospital, 966 Huaihai Middle Road, Shanghai 200031, China
[b] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China
[c] Clinical Medicine Department, Medical College, Jiangsu University, Zhenjiang, China
[d] Department of Endocrinology, Shanghai Xuhui Central Hospital, Shanghai, China

ARTICLE INFO

Article history:
Received 1 June 2008
Accepted 25 September 2008
Available online 2 October 2008

Keywords:
1,5-Anhydroglucitol
LC–MS/MS
Glycemic monitoring marker
Human plasma

ABSTRACT

Previous studies have shown that plasma 1,5-anhydroglucitol (1,5-AG) is markedly reduced among diabetic patients and therefore serves as a sensitive marker for short-term glycemic control. The current study describes the development of the liquid chromatography negative ion electrospray tandem mass spectrometry (LC–MS/MS) method to measure 1,5-AG in human plasma. The samples were pre-treated with protein precipitation and an isotope-labeled internal standard was used. Chromatographic separation was achieved on amide column (150 mm × 2.0 mm i.d., 5 μm) followed by detection with multiple reaction monitoring mode. Linearity, accuracy, precision, recovery, matrix effect, and stability were evaluated during method validation over the range of 1–50 μg/mL. The validated method has been clinically applied among 159 type 2 diabetic patients and 290 control subjects. A marked reduction in 1,5-AG levels among the diabetic patients and significant between-gender difference in nondiabetic subjects were observed.

1. Introduction

As a natural polyol, 1,5-anhydroglucitol (1,5-AG) is competitively inhibited by glucose for renal reabsorption [1]. The level of 1,5-AG is stable among normal subjects but significantly decreases among diabetic patients. This compound has been proposed and validated early in Japan [2–7] and recently in the US [8–12] as a marker for short-term glycemic control. The assay of 1,5-AG (GlycoMark™) was granted marketing clearance by the US Food and Drug Administration (FDA) in 2003, with the enzymatic method operated on Hitachi 917 chemistry analyzer [13].

It has been well established that improved glycemic control helps to prevent cardiovascular complications in diabetic patients [14–16]. The currently available markers for glycemic control are glycated haemoglobin (HbA1C), fructosamine (FA), capillary glucose monitoring (self-monitored blood glucose, SMBG) and continuous glucose monitoring (CGMS). These are widely used in clinical practices. Though HbA1C is a generally recognized gold-standard method, reflecting mean glucose levels over the preceding 2–3 months, it demonstrates some limitations in delaying the modification of diabetic therapy and in differentiating postprandial hyperglycemia from fasting hyperglycemia [8,17]. FA is reflective of glycemia over a 1–2 week period, but it is prone to day-to-day variability [18]. SMBG provides the real-time assessments needed in identifying hypoglycemia and hyperglycemia and in adjusting therapy, but its application may be limited due to the inconvenience, physical discomfort, disability and cost [19]. Finally, CGMS can help identify the fluctuations and trends that would otherwise go unnoticed with standard HbA1C tests and intermittent finger stick measurements. However, current devices for continuous glucose monitoring purpose are still invasive and costly [20].

Serving as a marker for glycemic excursions, often in postprandial state, 1,5-AG is sensitive and rapidly responds to changes in glycemia [2,21], more robustly than HbA1C and FA [11]. Moreover, it has been shown to reflect the therapy effect of antidiabetic medication in a dynamic manner [2,22,23], and is able to accurately provide a better prediction of the rapid changes in glycemia than HbA1C or FA [2,9,11,12,21]. It is also more closely associated with glucose fluctuations and postprandial glucose [21]. Thus, 1,5-AG is suggested as a complementary indicator of postprandial hypoglycemia to HbA1C in clinical practice [8,11].

In many cases, serum or plasma 1,5-AG levels were assayed by an enzymatic method using a series of enzymes including glucokinase and pyranose oxidase to remove interference and then produce colorimetrical product [13,24,25]. This method is sensitive and precise,

* Corresponding author. Tel.: +86 21 54037810x2130.
E-mail address: chen-yu@online.sh.cn (C. Yu).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.09.033

but it can also be disadvantageous for its expensive multi-enzyme reagents. Likewise, the results can easily be affected by endogenous interference such as glucose, conjugated bilirubin and hemoglobin [25], myo-inositol [26], maltose [27]. Therefore, other methods for plasma/serum or urine 1,5-AG measurement using chromatography techniques such as HPLC, GC/MS and LC/MS were reported [26,28–30]. These methods may involve either time-consuming derivatization or laborious sample preparation procedures.

The purpose of this study is to develop an alternative LC–MS/MS-based method for the simple and rapid determination of plasma 1,5-AG. The method is successfully applied to determine 1,5-AG levels in real plasma samples among type 2 diabetic patients and healthy subjects in a clinical study. It has been validated and proven to be simple in terms of sample preparation, requires inexpensive analytical reagents, and has high throughput in the turnaround.

2. Experimental

2.1. Chemicals and reagents

The chemicals used were obtained from the following: 1,5-anhydro-D-glucitol (1,5-AG, purity 99.5%) from Sigma–Aldrich Inc. (St. Louis, MO, USA), internal standard (IS) 1,5-anhydro-D-[UL-$^{13}C_6$] glucitol (98 atom% ^{13}C) from Omicron Biochemicals Inc. (South Bend, IN, USA), and HPLC-grade acetonitrile and methanol from Tedia Company Inc. (Fairfield, OH, USA). All other reagents were of analytical grade. Double distilled water was used throughout the study.

2.2. LC–MS/MS analysis

The Shimadzu HPLC system (Kyoto, Japan), which consists of two LC-20AD pumps, a SIL-HTC autosampler and an online DGU-20A3 vacuum degasser, was used. Chromatographic separation was achieved on the analytical column CAPCELL PAK NH2 UG80 S5 column (150 mm × 2.0 mm i.d., 5 μm, Shiseido) coupled with the guard column Phenomenex NH2 (4.0 mm × 3.0 mm i.d. 5 μm). The mobile phase was composed of acetonitrile–water (80:20, v/v) and was subsequently eluted at a flow rate of 0.3 mL/min. The total run time was 5 min.

A triple quadrupole tandem mass spectrometer API 3000 (Applied Biosystems/MDS Sciex, Toronto, Canada) equipped with Turbo Ionspray source was operated in negative ionization mode. Multiple reaction monitoring (MRM) analysis was applied to detect ion transitions at *m/z* 163 > 101 and 169 > 105 for 1,5-AG and IS, respectively. The ion spray voltage was set at −4500 V and the source temperature at 450 °C. The collision activated dissociation (CAD) was set at 7 using nitrogen as collision gas. Analyst 1.4 software was used for instrument control and data acquisition.

2.3. Sample preparation

A total of 50 μL of plasma and 5 μL of IS solution (200 μg/mL aqueous solution) were added to a 1.5-mL Eppendorf tube. This was followed by the addition of 200 μL of methanol and vortex-mixing for 10 s. The mixture was centrifuged at 16,000 × *g* for 3 min. The supernatant was diluted five fold with acetonitrile, and then 5 μL of the sample was injected to the LC–MS/MS system.

2.4. Calibration standards and quality control (QC) samples

Stock solutions of 1,5-AG were prepared at 1.0 mg/mL and IS at 0.2 mg/mL with water. Working solutions of 1,5-AG were obtained by serial dilution from a stock solution with water. Pooled human plasma with low 1,5-AG concentration (less than 5 μg/mL) was collected for the preparation of calibration standards and quality control samples. Then the working solutions were spiked to pooled plasma to obtain calibration standards of 1, 2.5, 5, 10, 25, and 50 μg/mL. QCs were prepared in the same way at concentrations of 3, 15, and 40 μg/mL. The standard samples and QCs were prepared for each analytical batch along with the unknown samples. All stock solutions and working solutions were stored at 4 °C.

2.5. Method validation

The method was validated by verifying linearity, lower limit of quantification (LLOQ), intra- and inter-assay precision and accuracy, matrix effect and recovery, and stability.

The linearity for 1,5-AG was evaluated over the range of 1–50 μg/mL. Calibration curves with six levels were prepared by adding serial diluted working standards to pooled blank plasma. A linear regression model with $1/x$ weighted factor was constructed based on the measured peak area ratio of 1,5-AG to the internal standard versus the nominal concentration. LLOQ was the lowest concentration of analyte measured with acceptable accuracy and precision (% bias and CV less than 20%).

QCs at three concentration levels (3, 15 and 40 μg/mL) were analyzed to evaluate intra- and inter-assay precision and accuracy of the method with six replicates for each of the three randomized batches. Precision was expressed as CV% for replicate measurements and accuracy (%) by the percentage of deviation between nominal and calculated concentrations.

The matrix effect and recovery were assessed by comparing the peak areas of 1,5-AG from blank plasma, the neat QC standards, and the standards spiked before and after extraction in six different lots of plasma at three concentration levels.

The stock solution stability of 1,5-AG and IS was tested at 4 °C for 30 days. The freeze–thaw stability was evaluated by analyzing the QC plasma samples at three concentration levels after three cycles from −20 °C to room temperature. The autosampler stability was assessed by keeping the processed QC samples at room temperature for 24 h. Furthermore, the plasma samples were analyzed for bench top stability after storage at room temperature for 6 h. Long-term storage stability was evaluated by freezing the QC samples at −20 °C for a month, then comparing the concentrations with those of QCs before the storage period.

2.6. Clinical application

The developed method was applied to determine the plasma concentrations of 1,5-AG from 267 young healthy subjects (82 males and 185 females) for routine physical examination, 23 aged nondiabetic subjects (9 males and 14 females) and 159 patients with type 2 diabetes (59 males and 104 females). The young healthy subjects and aged nondiabetic subjects were served as control group. Blood was collected from enrolled subjects after fasting overnight. Plasma was then separated by centrifugation at 3000 × *g* for 10 min, and then stored at −20 °C until analysis. The study was approved by the Ethics Committee of Shanghai Xuhui Central Hospital. Each subject was informed of the purpose of the study, and informed consents were obtained.

2.7. Statistics analysis

Data were analyzed by using SPSS for Windows 11.5 Standard Version (SPSS Inc. Chicago, IL). The mean, range, median, and SD were calculated for all subjects on the basis of gender and age.

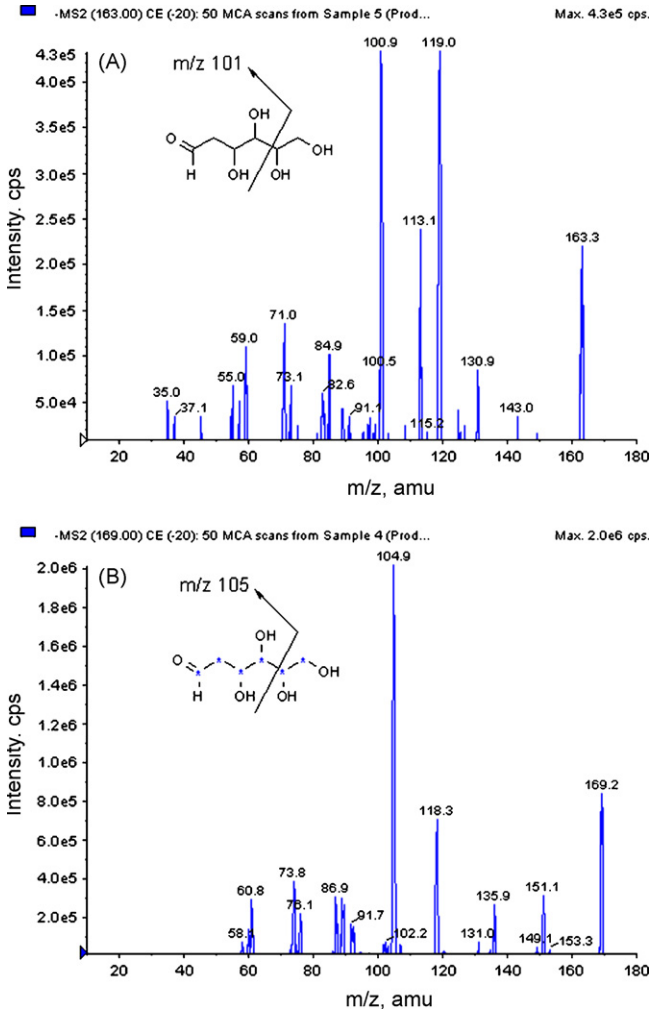

Fig. 1. MS/MS product ion spectra of (A) 1, 5-AG and (B) internal standard $^{13}C_6$-1, 5-AG isotope-labeled atoms were marked as blue. The deprotonated molecule $[M–H]^-$ of 1,5-AG and $^{13}C_6$-1,5-AG were detected at *m/z* 163.3 and *m/z* 169.2, respectively, with major product ions, at *m/z* 100.9 and *m/z* 104.9, which were chosen to detect ion transitions in MRM mode.

A normal distribution analysis was verified by the one sample K–S nonparametric tests based on gender. The difference in data between control subjects and diabetic patients, males and females were compared using one-way analysis of variance. Statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. Optimization of LC–MS/MS conditions

Negative ion mode was selected to scan 1,5-AG with chemical standard solution. In the present study, to obtain the appropriate ionization mode in analysis, 1,5-AG and IS was scanned with both negative and positive ion mode by injection of standard solution to mass spectrometer. The more significant signal of deprotonated molecule $[M–H]^-$ at *m/z* 163 and a major product ion at *m/z* 101 were found in Q1 and product ion scan. Isotope-labeled $^{13}C_6$-1,5-AG was used as internal standard and its corresponding ions at *m/z* 169 and 105 were observed (Fig. 1). The optimization processes were performed by tuning source/gas and compound dependant parameters (including nebulizer gas, curtain gas, ionspray voltage, declustering potential, focusing potential, entrance potential, collision energy, and collision cell exit potential) at multiple reaction monitoring (MRM) mode to obtain the intensest signal. To our knowledge, no such methods for 1,5-AG using electrospray mass spectrometry was reported. Compared with the previous APCI–MS method [26], which involved many tedious procedures such as post-column addition of chloroform to enhance ionization efficiency, multiple steps in sample preparation, single quadrupole MS in detection, the ESI–MS/MS method may be advantageous in specificity and simplicity.

Chromatographic conditions were optimized to provide suitable retention times and a satisfactory peak shape. A typical condition of amide-based analytical column and acetonitrile/water-composed mobile phase was selected to perform the chromatographic separation of the naturally occurring polyol in plasma. It is difficult to employ liquid–liquid extraction or solid phase extraction for 1,5-AG due to its high hydrophilicity. Hence, a protein precipitation procedure with methanol for the sample preparation was adopted. Fig. 2 shows the representative chromatogram of 1,5-AG and IS at 3.5 min, with a turnaround of 5-min, which enables to assay more than 100 samples in a working day. Nowatzke and co-workers evaluated the GlycoMark 1,5-AG assay with enzymatic method run on Hitachi 917 analyzer, the enzyme reagents reaction required at least 10 min [13]. Feng and co-workers developed a novel fully enzymatic method with three reaction steps which involved more than 24 min in assay procedure [24]. Niwa and co-workers described a LC–MS method with a chromatographic separation run for 20 min [26]. Actually, the method we reported here has remarkable advantage in assay throughput.

1,5-AG is a high polarity compound with high concentration in plasma, and isotope dilution technique was used, so we adopt a protein precipitation procedure by addition of methanol. To reduce the contamination to the ion source of mass spectrometer, the supernatant was further diluted five times and a small volume (5 μL) was injected. Unlike the existing methods including time-consuming derivatization protocols [28,29] or multiple clean-up procedures [26], the present method involves an one-step and cost-effective process.

3.2. Linearity and LLOQ

A calibration curve was constructed by plotting 1,5-AG to the IS peak area ratio against 1,5-AG concentration using linear regression with $1/x$ weighting. A different lot of pooled plasma with low endogenous concentration (typically <5 μg/mL) was used for each of the three batches. Calibrators and QCs were prepared in such pooled plasma by addition of working solution corresponding to 1,5-AG concentrations of 0, 1, 2.5, 5, 10, 25, 50 μg/mL and 3, 15, 40 μg/mL. The background derived from endogenous concentration of pooled plasma was subtracted from calibration points and QC samples. Excellent linearity ($r > 0.999$) was observed over the quantification range of 1–50 μg/mL for 1,5-AG in human plasma. After validation using three continuous batches, the method exhibited acceptable results with %CV of 2.53% for slope, % bias of 0.35–2.33%, and %CV of 1.05–4.11% for the back calculated concentration of 1,5-AG calibrators (Table 1). The limit of detection (LOD) was about 0.1 μg/mL (3× S/N), and the lower limit of quantification (LLOQ) was 1 μg/mL, at which concentration 10 times of peak response signal to noise (10× S/N) was observed with acceptable and reproducible precision and accuracy. We have determined the 1,5-AG concentration in diabetic patients and normal subjects. The results ranged from 1.6 to 48.3 μg/mL. This is the reason why we choose the above linearity range for the method. The sensitivity of the method is sufficient to meet the

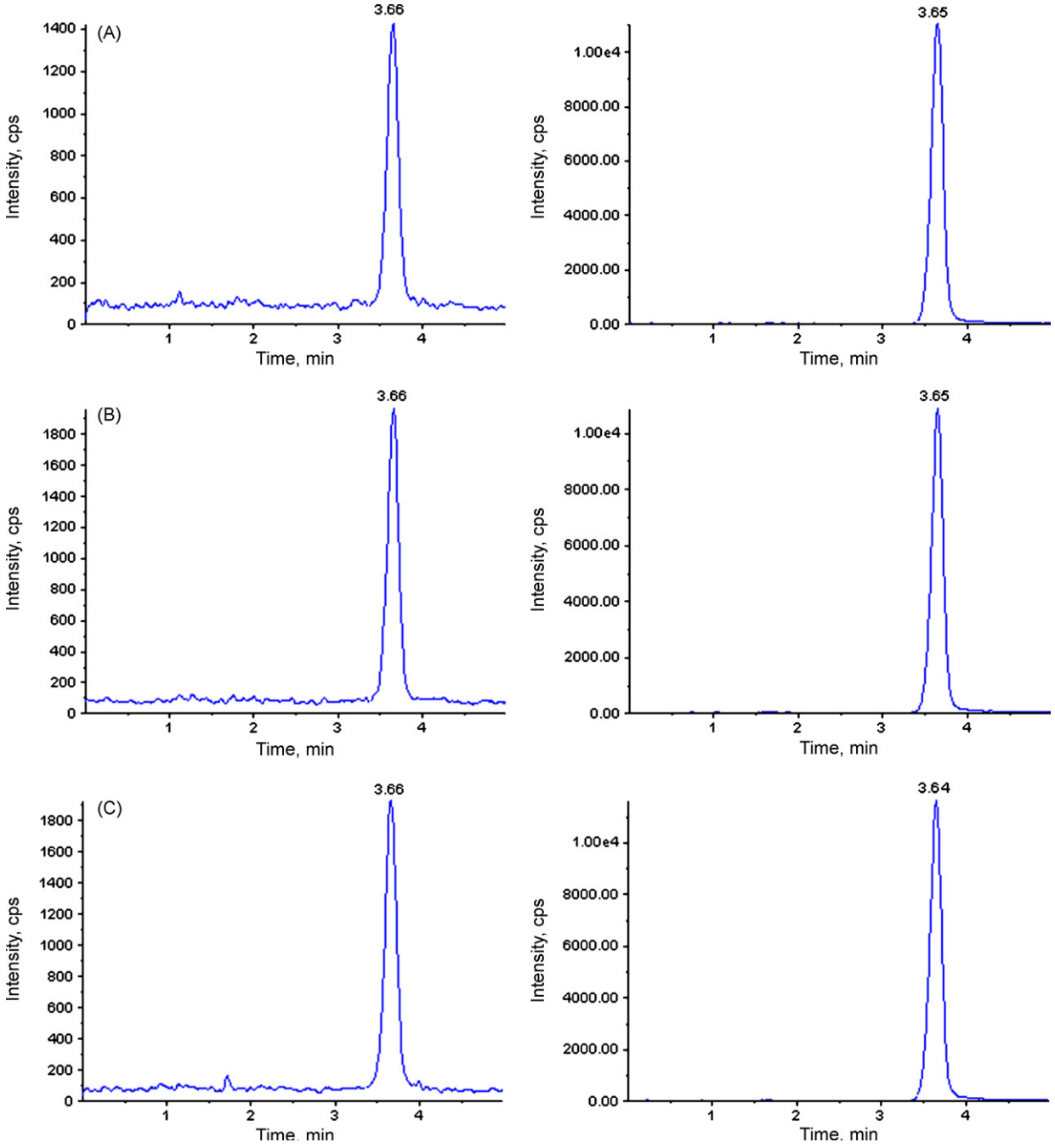

Fig. 2. Representative chromatograms of 1,5-AG (left) and internal standard (right) acquired from (A) Pooled blank plasma (1.95 μg/mL 1,5-AG), (B) Blank plasma spiked with 1 μg/mL 1,5-AG (LLOQ), and (C) Plasma collected from a diabetic patient (3.03 μg/mL 1,5-AG).

requirement for quantification of 1,5-AG in these target population.

3.3. Accuracy and precision

Intra- and inter-assay accuracy and precision were evaluated by assaying the QC samples. Table 2 summarizes the assay results of 1,5-AG QC data at four levels in human plasma. In this assay, the intra-assay precision was 6.37% or less, and the inter-assay precision was 8.75% or less. Moreover, the accuracy ranged from 96.2 to 103.1%, at LLOQ, low, middle, and high QC level. The results demonstrate that the values are within the acceptable criteria, and the method is sufficiently accurate and precise.

3.4. Recovery and matrix effect

To compare the detection response of 1,5-AG in plasma from different subjects or sources, recovery and matrix effect were evaluated by calculating the peak area ratio using six lots of blank plasma. Table 3 shows the summary of recovery and matrix effect results obtained from three QC levels. The mean recoveries of 1,5-AG at 3, 15, and 40 μg/mL were 103.8, 98.0 and 98.2%, respectively, with %CV less than 3.3%. The method demonstrates almost no matrix effect from biological material, with a mean peak area ratio ranging from 94.5 to 100.9% obtained from the post-extracted samples. No interferences from other compounds present in plasma were observed in the assay of the samples. To date, no significant interferences

Table 1
Mean inter-assay calibration curve results of 1,5-anhydroglucitol in human plasma ($n = 2$, for three batches).

Nominal concentration (μg/mL)	Calculated concentration (μg/mL)			Mean	CV (%)	Bias (%)
	Run 1	Run 2	Run 3			
1	1.04	0.98	1.05	1.02	3.66	2.30
2.5	2.43	2.55	2.49	2.49	2.41	0.35
5	4.85	5.15	5.09	5.03	3.15	0.59
10	10.14	9.82	9.34	9.77	4.11	2.33
25	25.1	24.35	24.13	24.53	2.08	1.90
50	49.93	50.65	50.96	50.51	1.05	1.03
Slope	0.0547	0.0543	0.0569	0.0553	2.53	
Intercept	0.329	0.152	0.109	0.197		
r	0.9998	0.9997	0.9991	0.9995		

CV, coefficient of variation.

Table 2
Accuracy and precision results for the 1,5-anhydroglucitol assay in human plasma ($n = 6$, for three batches).

Nominal concentration (μg/mL)	Intra-assay ($n = 6$)		Inter-assay ($n = 18$)	
	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
1	6.4	103.1	NA	NA
3	3.1	98.7	8.8	97.6
15	1.4	95.3	0.8	96.2
40	1.7	96.3	1.0	96.9

CV, coefficient of variation. NA, not applicable.

from endogenous substances were observed during the process of method validation and sample assay.

3.5. Stability

The 1,5-AG stabilities were investigated in autosampler, after three freeze–thaw cycles, on bench top and after a 3-month period of storage at −20 °C. The results were listed in Table 4, indicating that the analyte remained considerably stable under the above conditions, with the accuracy ranging from 99.3 to 105.5% and the %CV being less than 7.7%. The good stability of 1,5-AG simplified the precautions needed for laboratory manipulations during the assay procedures. In addition, stock solutions were shown to be stable at 4 °C for 30 days, which can be deduced by comparing the peak areas with those of freshly prepared solutions. These results are consistent with those obtained using GlycoMark assay, which showed that 1,5-AG remained stable after three freeze–thaw cycles, at 4, 22 and −80 °C for 7, 5 and 14 days, respectively [13].

3.6. Clinical application

The validated LC-MS/MS method has been applied to determine 1,5-AG concentrations in plasma from 159 patients with type 2 diabetes, 267 young healthy subjects, and 23 aged nondiabetic subjects. The results are summarized in Table 5. Values of 1,5-AG concentrations were normally distributed based on gender in healthy subjects as well as in the patient group. No significant age differences were noted between-sex in both investigated groups. The mean values of plasma 1,5-AG concentrations were significantly higher in men than in women among the young and aged nondiabetic control groups ($P < 0.001$). However, no substantial gender differences were noted among the diabetic patient group ($P > 0.05$). Among diabetic patients, 1,5-AG concentrations demonstrated an approximately 60% decline in females and 77% in males as compared to the corresponding populations in the nondiabetic control groups. Overall, our study's findings are comparable with

Table 3
Recovery and matrix effect of 1,5-anhydroglucitol in human plasma ($n = 6$).

Nominal concentration (μg/mL)	Recovery		Matrix effect	
	Peak area ratio (%)	CV (%)	Peak area ratio (%)	CV (%)
3	103.8	3.3	94.5	4.9
15	98.0	2.7	99.0	0.9
40	98.2	1.3	100.9	1.8

CV, coefficient of variation.

Table 4
Stability of 1,5-anhydroglucitol in human plasma.

Nominal concentration (μg/mL)	Calculated concentration (μg/mL)	CV (%)	Accuracy (%)
Auto-sampler stability (room temperature for 24 after processing)			
3	3.0	4.6	100.9
15	15.4	0.5	102.7
40	40.6	1.3	101.6
Freeze–thaw stability (three cycles)			
3	3.1	7.7	103.7
15	15.8	6.4	105.5
40	41.7	1.9	104.2
Bench top stability (room temperature for 6 h)			
3	3.0	7.0	99.7
15	15.3	6.6	101.9
40	39.7	4.8	99.3
Long-term storage stability (−20 °C for 3 months)			
3	3.0	4.9	100.1
15	15.8	3.7	105.3
40	38.0	3.7	94.9

CV, coefficient of variation.

Table 5
1,5-Anhydroglucitol concentrations in diabetic patients (type 2), young healthy subjects, and aged nondiabetic subjects by gender group.

Group	Gender	n	Age (years)	1,5-AG (μg/mL)
Diabetic patients	Female	104	71.54 ± 9.90	7.62 ± 5.52**
	Male	55	75.53 ± 9.08	6.59 ± 5.20**
Young healthy subjects	Female	185	29.59 ± 6.12##	18.96 ± 5.71
	Male	82	31.11 ± 8.46##	28.97 ± 7.57▽▽
Aged nondiabetic subjects	Female	14	81.71 ± 11.87	19.98 ± 6.73
	Male	9	70.89 ± 14.15	27.26 ± 10.92▽

** $P < 0.001$ vs. young healthy subjects and aged nondiabetic subjects.
$P < 0.001$ vs. diabetic patients and aged nondiabetic subjects.
▽ $P < 0.05$ vs. females within group.
▽▽ $P < 0.01$ vs. females within group.

the results previously reported in 1,5-AG measurement based on the population in different regions [9,13,24]. We therefore hope that the determination of plasma 1,5-AG concentration in a large population of Chinese healthy subjects and diabetic patients will aid in the construction of reference intervals for this target population. Reference intervals of 1,5-AG should be set separately for males and females in clinical application because considerable gender-related difference was observed among control group. The results suggested that 1,5-AG level is closely correlated to diabetes and gender, but not to age. Moreover, a considerable inter-individual variation in 1,5-AG concentration was noted, which may limit 1,5-AG's suitability as a first-line screening tool for diagnosing diabetes [8].

4. Conclusion

A liquid chromatography-negative ion electrospray tandem mass spectrometry method was developed for the quantification of plasma 1,5-anhydroglucitol (1,5-AG), which is a validated marker for short-term glycemic monitoring in the management of diabetes in clinical practice. Isotope $^{13}C_6$-1,5-AG was added as internal standard. A simple protein precipitation procedure was adopted for sample preparation and a turnaround of 5 min for sample assay was achieved. No interference with the LC–MS/MS method from endogenous substances has been observed. The validated method has been successfully applied to the determination of 1,5-AG levels in the plasma samples which collected from 159 diabetic patients, 267 young healthy subjects, and 23 aged nondiabetic subjects. This is helpful in the creation of reference intervals for the target population and is ideally suited for glycemic management in clinical practice.

Acknowledgements

The authors thank Dr. Xiaochuan Li for his suggestions to improve the manuscript. This study was financially supported by the research grant SHXH20070108 from the Health Bureau of Xuhui District, Shanghai, China.

References

[1] T. Yamanouchi, Y. Tachibana, H. Akanuma, S. Minoda, T. Shinohara, H. Moromizato, H. Miyashita, I. Akaoka, Am. J. Physiol. 263 (1992) E268.
[2] T. Yamanouchi, N. Ogata, T. Tagaya, T. Kawasaki, N. Sekino, H. Funato, L. Akaoka, H. Miyashita, Lancet 347 (1996) 1514.
[3] T. Yamanouchi, Y. Akanuma, Diabetes Res. Clin. Pract. 24 (Suppl.) (1994) S261.
[4] A.L. Frattali, B.A. Wolf, Clin. Chem. 40 (1994) 1991.
[5] M. Emoto, T. Tabata, T. Inoue, Y. Nishizawa, H. Morii, Nephron 61 (1992) 181.
[6] T. Yamanouchi, S. Minoda, M. Yabuuchi, Y. Akanuma, H. Akanuma, H. Miyashita, I. Akaoka, Diabetes 38 (1989) 723.
[7] H. Akanuma, K. Ogawa, Y. Lee, Y. Akanuma, J. Biochem. 90 (1981) 157.
[8] K.M. Dungan, Expert Rev. Mol. Diagn. 8 (2008) 9.
[9] T.M. Nguyen, L.M. Rodriguez, K.J. Mason, R.A. Heptulla, Pediatr. Diabetes 8 (2007) 214.
[10] M. Dworacka, E. Wender-Ozegowska, H. Winiarska, M. Borowska, A. Zawiejska, M. Pietryga, J. Brazert, K. Szczawinska, T. Bobkiewicz-Kozlowska, Diabet. Med. 23 (2006) 171.
[11] K.M. Dungan, J.B. Buse, J. Largay, M.M. Kelly, E.A. Button, S. Kato, S. Wittlin, Diabetes Care 29 (2006) 1214.
[12] M. Dworacka, H. Winiarska, Dis. Markers 21 (2005) 127.
[13] W. Nowatzke, M.J. Sarno, N.C. Birch, D.F. Stickle, T. Eden, T.G. Cole, Clin. Chim. Acta 350 (2004) 201.
[14] M. Brownlee, I.B. Hirsch, JAMA 295 (2006) 1707.
[15] T. Kawasaki, N. Ogata, H. Akanuma, T. Sakai, H. Watanabe, K. Ichiyanagi, T. Yamanouchi, Metabolism 53 (2004) 583.
[16] DCCT/EDIC Study Group, JAMA, 287 (2002) 2563.
[17] American Diabetes Association, Diabetes Care 30 (Suppl. 1) (2007) S4.
[18] J.B. Buse, J.L. Freeman, S.V. Edelman, L. Jovanovic, J.B. McGill, Diabetes Technol. Ther. 5 (2003) 355.
[19] J. Davidson, Am. J. Med. 118 (2005) 27S.
[20] H. Hanaire, Diabetes Metab. 32 (2006) 534.
[21] J.B. McGill, T.G. Cole, W. Nowatzke, S. Houghton, E.B. Ammirati, T. Gautille, M.J. Sarno, Diabetes Care 27 (2004) 1859.
[22] A.C. Moses, P. Raskin, N. Khutoryansky, Diabet Med 25 (2008) 200.
[23] T. Yamanouchi, T. Sakai, K. Igarashi, K. Ichiyanagi, H. Watanabe, T. Kawasaki, Diabet. Med. 22 (2005) 980.
[24] F. Jing, L. Jun, Y. Wang, M. Zhu, Z. Yong, X. Fei, J. Zhang, Appl. Biochem. Biotechnol. 150 (2008) 327.
[25] Y. Fukumura, S. Tajima, S. Oshitani, Y. Ushijima, I. Kobayashi, F. Hara, S. Yamamoto, M. Yabuuchi, Clin. Chem. 40 (1994) 2013.
[26] T. Niwa, L. Dewald, J. Sone, T. Miyazaki, M. Kajita, Clin. Chem. 40 (1994) 260.
[27] Y. Fukumura, S. Oshitani, Y. Ushijima, I. Kobayashi, Y. Murahashi, K. Kajiwara, T. Jinnouchi, S. Tanaka, S. Ichimura, S. Tajima, et al., Clin. Chem. 38 (1992) 2553.
[28] M. Katayama, Y. Matsuda, K. Kobayashi, S. Kaneko, H. Ishikawa, Biomed. Chromatogr. 20 (2006) 440.
[29] E. Pitkanen, Clin. Chim. Acta 251 (1996) 91.
[30] S. Tanaka, K. Nakamori, H. Akanuma, M. Yabuuchi, Biomed. Chromatogr. 6 (1992) 63.